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Vitamin D Sufficiency: How Should it be Defined and what are its Functional Indicators?

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It has been more than three decades since the first assay assessing circulating 25 (OH)D in human subjects was performed. That publication as well as several that followed it defined "normal" nutritional vitamin D status in human populations. Recently, the wisdom by which "normal" circulating 25 (OH)D levels in human subjects were assigned in the past has come under question. It appears that sampling human subjects, who appear to be free from disease, and assessing "normal" circulating 25 (OH)D levels by plotting a Gaussian distribution is grossly inaccurate. There are many reasons why this method is inaccurate, including race, lifestyle habits, sunscreen usage, age, latitude, and inappropriately low dietary recommendations for vitamin D. For instance, a 400 IU/day. AI for vitamin D is insignificant when one considers that a 10-15 minute whole body exposure to peak summer sun will generate and release up to 20,000 IU vitamin D_3 into the circulation. Recent studies, which orally administered up to 10,000 IU/day vitamin D_3 to human subjects for several months, have successfully elevated circulating 25 (OH)D levels to those observed in individuals from sun-rich environments. Further, we are now able to accurately assess sufficient circulating 25 (OH)D levels utilizing specific biomarkers instead of guessing what an adequate level is. These biomarkers include intact parathyroid hormone (PTH), calcium absorption, bone mineral density (BMD), insulin resistance and pancreatic beta cell function. Using the data from these biomarkers, vitamin D deficiency should be defined as circulating levels of 25 (OH)D $\leq$ 30 ng/mL. In certain cases, such as pregnancy and lactation, significantly higher circulating 25 (OH)D levels would almost certainly be beneficial to both the mother and recipient fetus/infant.

Key Words: Vitamin D, 25-hydroxyvitamin D, Parathyroid hormone, Vitamin D requirement, Bone mineral density, Calcium absorption

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INTRODUCTION

What is a normal circulating level of 25 (OH)D in humans? This is an easy question to answer. You simply gather a diverse population of subjects that are asymptomatic for disease, assess circulating 25 (OH)D and plot the data using a Gaussian distribution. You now have normative data to assess circulating 25 (OH)D in that population. This is how Haddad and Chyu[1] performed their assessment of 25 (OH)D status more than thirty years ago. The data from their initial study is summarized in Table 1. They referred to their normal volunteers as the normal population for circulating 25 (OH)D levels. Their study also presented a group of lifeguards that had circulating 25 (OH)D levels 2.5 times that of the "normals." Countless similar studies have been performed in the ensuing decades reiterating the same conclusion. I, however, will interpret the original Haddad data differently. I conclude that the 25 (OH)D levels in the lifeguards are normal and the "normals" are actually vitamin D deficient. The justification for this conclusion will be forthcoming in this text.

Table 1. Original Assessment of Nutritional Vitamin D Status Circa 1971

Group	No.	Age (years)	Consumption of Vitamin D Weekly (units)	Weekly Exposure to Sunlight (hours)	Plasma 25 (OH)D (nmol)
Normal Volunteers	40	30.2±12.9	2230±1041	8.8±6.1	68.3±29.5
Biliary Cirrhosis	4	1.5-55	2500 (est.)	--------	16±6.5*
Lifeguards	8	18.5±2.0	2895±677	53.0±10.3	161±21.8*

*p<0.00
+values represent mean±SD

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CUTANEOUS GENERATION OF VITAMIN D_3

For all practical purposes, vitamin D does not naturally occur in foodstuffs that humans eat. There are exceptions-such as oily fish and fish liver oil; however, for the most part, vitamin D does not exist in the human foodchain. The fact is, from an evolutionary standpoint, humans did not require vitamin D in their foodchain because over millions of years humans, along with many animal species, evolved a photosynthetic mechanism in their skin to produce large amounts of vitamin D_3. Thus, our skin is part of the vitamin D endocrine system and vitamin D_3 is really a preprohormone.

Approximately 50,000 years ago, small bands of people, almost certainly darkly pigmented, migrated gradually from sub-Saharan Africa to eventually populate more northern climates. This migration resulted in a profound evolutionary adaptation, a gradient in skin pigmentation loss to the point of almost total depigmentation as evidenced by northern European populations. Why would this dramatic change have occurred so rapidly? The most obvious answer is to maximize the limited sunlight exposure as occurs when moving north from the equator. Darkly pigmented individuals in an equatorial environment literally would be bathed in intense sunlight year round, and thus, vitamin D_3 production would not be a problem. However, as these darkly pigmented individuals migrated to a northern sun-restricted environment, they would rapidly become vitamin D-depleted, with the resulting mobility and reproductive problems associated with deficiency. For humans to survive in this new northern environment, skin depigmentation had to occur. Eskimos are an exception to this as they retain significant pigmentation. However, the Eskimos' diet is unique in that it contains significant levels of vitamin D_3 due to the fat and oily fish content.

The cutaneous generation of vitamin D_3 in humans has been well characterized both *in vitro* and *in vivo*.[2,3] Vitamin D_3 is produced in the skin from 7-dehydrocholesterol (7-DHC).2) 7-DHC is distributed throughout the epidermis and dermis with highest concentrations in the stratum spinosum and stratum basale.4) Exposure of skin to sunlight, specifically to the UVB range of the spectrum (290-315 nm) results in the photolytic convertsion of 7-DHC to previtamin D_3. Previtamin D_3 is transformed to vitamin D_3 by a thermally induced isomerization.[2] The production of vitamin D_3 is thought to be regulated by the amount of UVB light reaching the 7-DHC and not by hormonal feedback.[5] It is interesting that vitamin D intoxication has never been reported because of excessive exposure to sunlight. How the production of vitamin D_3 is limited in face of excessive UV irradiation and a continuous supply of the precursor 7-DHC can be explained.[2] On exposure to excessive sunlight, previtamin D_3 is transformed not only into vitamin D_3, but also into lumesterol or tachysterol, which are biologically inactive and thus, reduce the amount of previtamin D_3. It is also known that excess sunlight can degrade vitamin D_3 into inert photoproducts, including suprasterols I and II.[2] A number of other natural factors can limit or regulate the cutaneous production of vitamin D_3, including aging,[6] increased melanin pigmentation,[4,7] and season and latitude.[8] Of course, clothing and sunscreen also will eliminate the cutaneous generation of vitamin D_3.[9,10]

ALL SUNLIGHT IS NOT EQUAL

Growing up in northern Ohio, I can remember being told to go outside on a sunny, midwinter day and the sunshine on my face would provide me with the vitamin D that I required. This statement is a gross misconception on two counts. Webb, *et al.*,[8] first demonstrated *in vitro* that a UVB irradiation threshold of 20 mJ/cm^2 is required to induce the transformation of 7-DHC to previtamin D_3. This *in vitro* study soon was confirmed by Matsuoka, *et al.*,[3] *in vivo* using human subjects. Matsuoka, *et al.*, demonstrated that a UVB irradiation threshold of 18 mJ/cm^2 was required to induce vitamin D_3 production and its subsequent release into the circulation (Fig. 1).

These data also demonstrate that further increases in UVB energy delivered caused an exponential increase in the rate of production and release into the circulation. Further work by Matsuoka, *et al.*,[9] demonstrated what

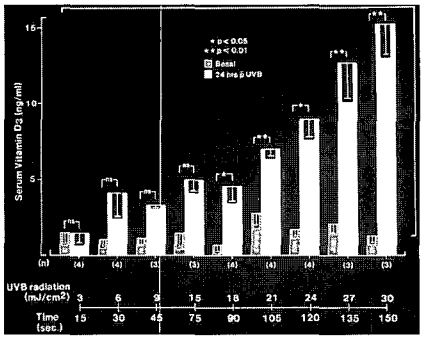

Fig. 1 Effect of graded doses of whole body UVB irradiance in untanned, healthy white subjects with skin type III. Results are expressed as mean±SEM. Time represents exposure period in phototherapy unit. From reference (3).

portions of the human body should be exposed to *UV* light to maximize *in vivo* vitamin D_3 production (Fig. 2). First, these data demonstrate just how effective sunscreen is at blocking cutaneous vitamin D_3 production. Secondly, the data clearly demonstrate that minimal skin exposure (arms and face) will result in a minimal production of vitamin D_3. This limited cutaneous production of vitamin D_3 is further exacerbated by increasing cutaneous melanin content due to race (Fig. 3).[7] The relevance of these findings to public health is clear. The exposure level of 18-20 mJ/cm^2 is not generally reached during the winter in northern United States above latitude 40°. For example, in Boston (42 degrees N latitude), the accumulated daily UVB solar irradiance (from 11:30 a.m. to 2:30 p.m. EST) remains below 20 mJ/cm^2 from November through February. Thus, a Caucasian individual in a bathing suit outside on a sunny January day in Boston would not produce endogenous vitamin D_3. Further, even in the summer with only one's arms and face exposed, minimal endogenous vitamin D_3 production would be achieved in that individual. In the African-American population, the situation is far worse.

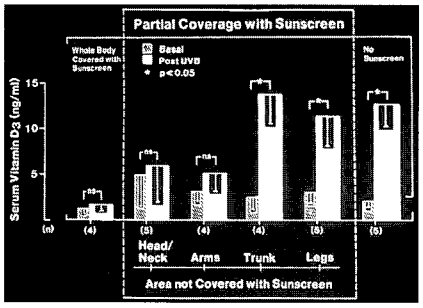

Fig. 2 Regional synthesis and release of vitamin D_3 in untanned young white subjects with skin type III. Sunscreening agent (SPF 15) was applied 1 hr before exposure to 27 millijoule/cm^2 of *UVB* irradiance. Vitamin D_3 levels were monitored at baseline and 24 hours post exposure. From reference (9).

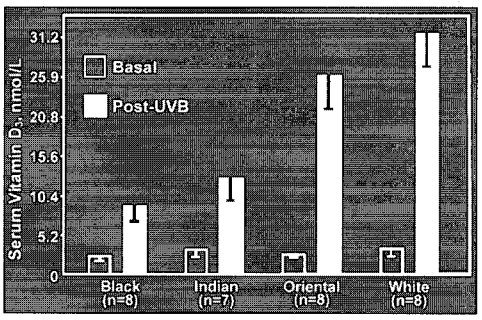

Fig. 3 Circulating vitamin D_3 response in the major racial groups following whole body exposure to a single fixed *UVB* radiation dose of 27 mJ/cm^2. Vitamin D_3 levels were monitored at baseline and 24 hours post exposure. From reference (7).

The question is then, how much vitamin D_3 do humans generate *in vivo* through solar exposure? Several investigators have addressed this question, and the answer has been fairly consistent.[3,5,11,12] Using synthetic *UV* sunlamps, a total body minimal erythemic dose (MED) can be determined for a given individual by graded exposure of small areas of the back to increases doses of *UVB*. Twenty-four hours later the individual returns and the least amount of exposure that causes an erythemic response (pinkening of the skin) is the MED for that individual. What does an MED mean from a practical sense? A Caucasian individual in a bathing suit who is not tanned would receive a total body MED from approximately 10-12 minutes of peak July summer sun (11:30 a.m. to 2:30 p.m. EST) in Boston. If you are an Asian Indian, that same MED could take perhaps 30 minutes of exposure, and if you are a very darkly pigmented African-American, it could be 120 minutes of exposure.

How much endogenous vitamin D_3 does a single MED produce? Several investigators, as mentioned earlier, have addressed this question. The method for the determination is quite straightforward. One simply compares circulating levels of vitamin D_3 at various time points from individuals provided a known oral supplement of vitamin D_3 to those receiving an MED of *UVB* (3,5,11-13). When this comparison is made, it is clear that a total body MED will release approximately 10,000 -20,000 IU vitamin D_3 into the circulation within 24 hours of exposure. Remember, of course, that the exposure time required to achieve that MED is highly dependent on skin pigmentation. Thus, at least in Caucasians, an intense but very brief sun exposure causes the release of a "large amount" of vitamin D_3 into the circulation. The "large amount" is defined, of course, against the current. AI of 400 IU/day for vitamin D.

WHAT IS THE NORMAL LEVEL OF CIRCULATING 25 (OH)D IN HUMANS?

What constitutes a normal circulating level of 25 (OH)D in humans has been an amazingly complex question to answer. On the surface, it appears like an easy task to perform. Simply obtain blood specimens from a diverse, apparently healthy population, assess circulating 25 (OH)D and plot the data using a Gaussian distribution. This design was used in the very first study performed more than three decades ago by Haddad and

Chyu[1] and has been used in countless studies since.

I propose that this design of defining "normal" circulating 25 (OH)D is flawed. To properly define "normal" 25 (OH)D status in humans, would it not make more sense to obtain "normal subjects" who are sunbathers, fieldworkers, construction workers or other individuals who work outside in scant clothes without sunblock? Using "normal subjects" to establish circulating 25 (OH)D levels who spend no time outside, wear sunblock and/or have dark skin a northern latitude is illogical and is akin to establishing "normal" estrogen levels on a population of women who are postmenopausal. Humans did not evolve in the artificial, sun paranoia environment that many of us live in today, so "normal" with respect to circulating 25 (OH)D levels is problematic. In sun-rich environments, circulating 25 (OH)D ranges from 135-225 nmol (54-90 ng/mL).[1,14,15] We must be very careful how we define "normal" with respect to circulating 25 (OH)D.

A more serious problem is how we define nutritional vitamin D deficiency in the human population. Again, during most of the past thirty years, deficiency has been defined in terms of a Gaussian distribution using sun-deprived "normal subjects" as a template, which-again, is illogical. Fortunately, in recent years investigators have begun to define nutritional vitamin D deficiency, circulating 25 (OH)D levels, using various biomarkers. These biomarkers include calcium homeostatic indicators PTH, calcium absorption and bone mineral density (BMD).[16-20] Recently, non-calcium homeostatic factors such as insulin resistance and beta cell function have been added to the list of 25 (OH)D-linked biomarkers.[21]

What do these biomarkers of 25 (OH)D function tell us about a set-point for the onset of nutritional vitamin D deficiency? Let us begin with PTH since a significant amount of work has been done with this biomarker. Many studies have shown the significant, inverse relationship between circulating 25 (OH)D and PTH.[16-18] The biological consequences, with respect to calcium homeostasis, due to the decline in nutritional vitamin D status are illustrated in Fig. 4.[22] This decline induces secondary hyperparathyroidism as can be observed in Fig. 5. Fig. 5 illustrates that secondary hyperparathyroidism may subside when circulating 25 (OH)D levels reach approximately 80 nmol (32 ng/mL). However, a more sophisticated mathematical model suggests that circulating 25 (OH)D has the ability to suppress PTH well above the 80 nmol range (Fig. 5). Similar results have been observed using calcium absorption studies.[19] When circulating 25 (OH)D drops below 80 nmol, calcium absorption is impaired (Fig. 6). It is logical that impaired calcium absorption and secondary hyperparathyroidism due to nutritional vitamin D deficiency would have an adverse impact on skeletal integrity. Ironically, a recent retrospective study details such a relationship.[20] Fig. 7 displays this remarkable relationship between circulating 25 (OH)D and BMD in several thousand patients. Further, a recent interventional study has demonstrated that circulating 25 (OH)D levels of 50 nmol did not maintain skeletal integrity as efficiently as levels above 80 nmol.[23] It comes as no surprise that

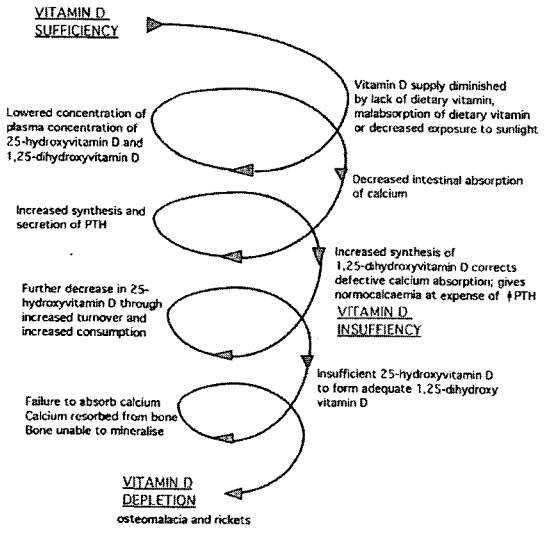

Fig. 4 Spiral of physiological developments as vitamin D deficiency progresses. From reference (22).

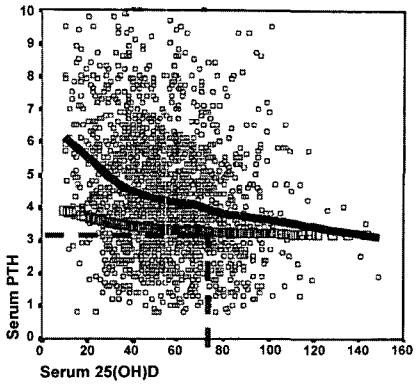

Fig. 5 Circulating PTH vs. circulating 25(OH)D concentrations in 1,741 patients, overlaid with the locally weighted regression and scatterplot smoothing (LOWESS) technique (dash-dot line) and exponential decay function fitted to the data (dashed line). The vertical dashed line indicates the point at which PTH concentrations theoretically attain the plateau value, based on the exponential function. PTH units are pmol/L. 25 (OH)D units are nmol/L. From reference (18).

circulating levels of 25 (OH)D of at least 80 nmol (32 ng/mL) are required to optimize this biomarker. It is quite apparent from Fig. 7 that a "normal range" for circulating 25 (OH)D set at 25 nmol (10 ng/mL) or even 37.5 nmol (15 ng/mL) will compromise skeletal integrity.

Biomarkers for defining circulating 25 (OH)D sufficiency need not only apply to calcium homeostasis. After all, vitamin D deficiency has been linked to a whole cadre of disorders including cancer, autoimmune diseases, musculoskeletal and neurological function, and diabetes.[24] A very recent study highlights the effect of nutritional vitamin D deficiency, insulin resistance and beta cell dysfunction.[21] This study utilized glucose-tolerant subjects and reported a highly significant positive relationship between circulating 25 (OH)D and insulin sensitivity and a highly negative relationship with respect to hypovitaminosis D and beta cell function. The authors concluded that subjects with hypovitaminosis D are at higher risk of insulin resistance and the metabolic syndrome. The data from this study strongly suggest that circulating levels of 25 (OH)D in excess of 80 nmol (32 ng/mL) would be beneficial with respect to insulin resistance and beta cell function.

Using the data described in the last few paragraphs, it is apparent that we no longer have to guess what a deficient level of circulating 25 (OH)D should be in a human subject. Clearly, there is no known disadvantage or harmful effect of maintaining a circulating 25 (OH)D level of at least 80 nmol but clearly there are risks at having levels less than this. Thus, nutritional vitamin D deficiency should be defined as <80 nmol (32 ng/mL) circulating 25 (OH)D. This is graphically displayed in Fig. 8. This figure is a simplified version of previous figures that had many stages of vitamin D status including deficiency, insufficiency, hypovitaminosis, sufficiency and toxicity.[25] Complicated staging charts may be fine for the researcher but are difficult to apply to clinical practice. Fig. 8 displays vitamin D deficiency, repletion and toxicity. The deficiency portion of this figure is based on biomarkers discussed previously in this text. However, the region between repletion and toxicity-hypervitaminosis D, remains blurred. I have arbitrarily set the toxic level at 250 nmol (100 ng/mL). This is a conservative estimate as true vitamin D toxicity is well beyond this point. A study has been completed that observed no harmful effects with 25 (OH)D levels of 250 nmol.[26] Additional studies on this topic are ongoing.

Let us discuss a hypothetical case here for those who do not view this deficiency setpoint as important. Let

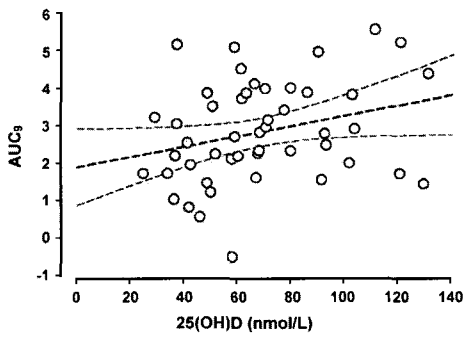

Fig. 6 Correlation of area under the curve with serum 25(OH)D concentrations in 48 measurements of calcium absorption in 34 postmenopausal women. The lines represent the least squares regression line through the data and its 95% confidence limits. From reference (19).

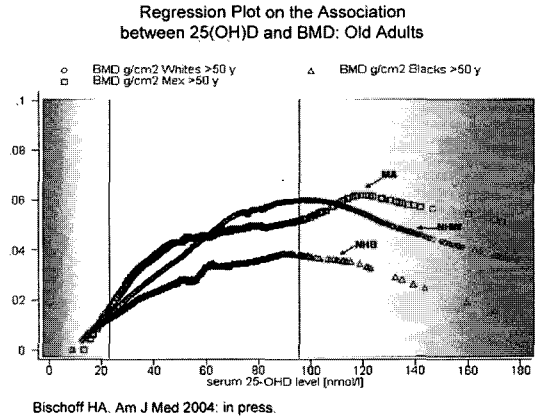

Fig. 7 Regression plot of bone mineral density change (g/cm^2) by 25 (OH)D level (nmol/L) in younger adults (20 to 49 years). Circles represent Caucasians, squares represent Mexican Americans, and triangles represent African Americans. From reference (20).

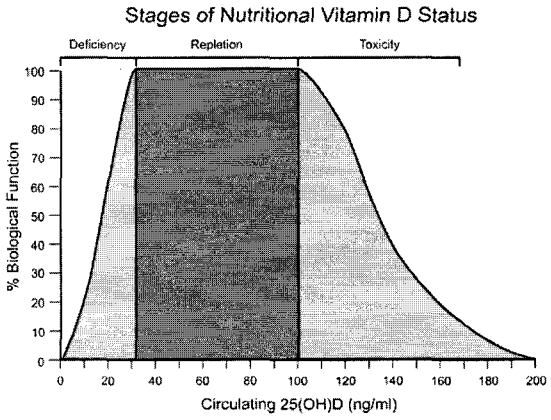

Fig. 8 Stages of nutritional vitamin D status. Concentrations in ng/mL can be converted to nmol/L using a multiplication factor of 2.5.

us say that an elderly woman is in a nursing home and she falls and fractures her hip. It is well known that vitamin D deficiency is a risk factor for hip fracture.[27-29] The woman's attending physician ordered a circulating 25 (OH)D level two months prior to the fracture and the value reported by the clinical laboratory at 25 nmol (12 ng/mL) and designated as "normal" based on Gaussian distribution data. The woman does not receive vitamin D therapy and subsequently falls and shatters her hip. Who would be legally to blame here? With the data presently in the literature about circulating 25 (OH)D and biomarkers, anyone reporting "normal levels" below 80 nmol would be at legal risk. Thus, I strongly suggest that assay manufacturers and clinical laboratories reporting results alter their "normal" circulating 25 (OH)D concentration to a minimum of 80 nmol. This would put the burden on the physician to treat the patient appropriately. If the laboratories do not adjust the deficiency level, the legal liability could go back as far as the assay manufacturers.

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